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メガウイルス科の DNA ポリメラーゼ遺伝子を標的とするアンプリコン解析

Amplicon analysis of Megaviridae DNA polymerase gene

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研究成果概要

This research was conducted in collaboration with Graduate School of Agriculture of Kyoto University. Megaviridae is a proposed family of eukaryotic viruses classified in the group of nucleocytoplasmic large double-stranded DNA viruses (NCLDV). They possess large genomes and atypical gene content as compared to other viruses. Genomic data suggest that the diversity of Megaviridae is vast and perhaps greater than that of cellular organisms. In the present work, we aimed at characterizing the diversity of Megaviridae at ecologically different sites using high-throughput sequencing analysis of PCR-amplified DNA polymerase family B (PolB) gene.

PolB is encoded in all available Megaviridae genome sequences but its high level of divergence makes it impossible to design a single PCR primer pair that would amplify all known Megaviridae PolB sequences. To overcome this limitation, we designed 82 degenerate primer pairs that target a conserved domain of PolB in 923 PolB sequences of isolated and environmental Megaviridae species. In silico tests demonstrated that this set of primer pairs specifically covers 97.4% of the 923 PolB sequences.

We tested all 82 primer pairs on 5 environmental DNA samples extracted from water samples collected at Osaka Bay, Japan Sea, Miyuki hot spring and a mangrove site of Ishigaki island. A preliminary analysis of the reads from Osaka Bay sample revealed that there are >5,000 OTUs for Megaviridae at 97%-identity clustering. Estimates of Megaviridae species diversity in these 5 environments will be presented along with the bioinformatics pipeline we developed to analysis this unique dataset.